

Ruben EXHIBIT #92

Department MOL. BIOL. - PROT. EXPR.

Subject 7/95 - 6/5/95

Name ANN KIM # 9

Address

43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701



0 73333 43648 8

75 Sheets
1 1/4" x 9 1/4"
4x4 Quad.

Ruben EXHIBIT 2092
Ruben v. Wiley et al.
Interference No. 105,077
RX 2092

2

WIAJ8 (95% + PAF10)
 Pg 151 Book 8 #23a

1/24/95

Spin HTDAN08185 bp + PAF10 in.
 1000 rpm HCl pH 8 - 8K 20min.

Equilibrate N. SO4 column with pH 8
 0.1M HCl

Apply Supernatant to Columns - Collect Flow
 Wash 30 ml pH 8 0.1M HCl - Collect pH 8
 Wash 30 ml pH 10 0.1M HCl - Collect pH 10
 Elute 5 ml pH 15 0.1M HCl - Collect pH 15
 Strip 30 ml pH 2 0.1M HCl - Collect pH 2.

Add 50ul of eluted collected material
 to 450ul H2O
 50ul 80% (15%) NaDOC
 75ul 50% TCA.

Mix well

Spin 5 min

Remove supernatant

Resuspended pellet in 15ul 0.2M NaOH / 15ul 2X Buff

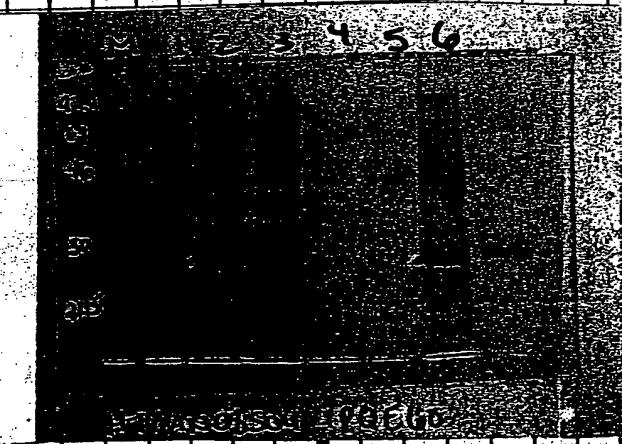
Heat 100°C 5 min

Run 20 ul on gel with Rainbow Marker.

on 12.5% gel 65min at 100V 1/2 hrs.

Stop 1/2 hr

De Stain 1/2 hr.



1000

1000

1000

1 Unconditioned
 2 Crude Extract
 3 flow
 4 pH 8
 5 pH 10
 6 pH 15
 7 pH 2
 8 50% NaOH
 9 50% TCA
 10 0.2M NaOH
 11 2X Buff
 12 80% NaDOC
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11/16 POE60

11/17 HPA08/85 bpt POE60

(pg 142 Book 8 #23(e))

2/2/95

11-16 - Recappy old column + Strip again
to try & Purify more.

Start Dialyzing ~~20 ml~~ in Dialysis
Tubing

3M 6n HCl / Hapes 5 hrs

1.5M 6n HCl / Hapes Over night.

Recappy & 3ml to Ni Sep Column, to Resinure
over column. of HPA08/85 bpt POE60.

2/3/95

Change Buffer

1M 6n HCl / Hapes 10 hrs.

0.5M 6n HCl / Hapes Over Weekend

Carrie will finish

2/16 - 2/10 Vacation

HPA08/85 bpt + POE60

2/3/95

Strip column that has ~~been~~ denatured. Protein in 2
strips in 1mm wide slot. Elution

Buffer: 50mM NaPO₄ pH 10

250mM Imidazole

300mM NaCl

10% Glycerol.

2 strips at 2.5ml each.

Run on Stacking gel with 1N H₂SO₄
marker - 8/2.5% gel 150V

Stack 1/2 hr.

Electro 1 hr.

4

1L-6 PGE60 (HTPANOB 85 bp + PQE60)

2/3/95

HTPANOB 85 bp + PQE60

31°

29

21

16

Store 4°C.

1L-6 PQE60 + PPO 2nd Column Extraction

1000 uL WT

100 uL

43

29

154

H1PANO8/H1PB411 - PD10 - PAZ

5

pg 152 Book 8 # 2240

1/26/95

Received Primers for
H1PANO8 & H1PB411
Reprotect 55°C O/N.

1/27/95

PCR Fragments

H1PB411 PAZ

9130 5'Bam.

1

3'Xba New.

1

10X dNTP

50

10X PCR

50

Taq

2

H₂O

397

DNA (cong/ful)

1

500ul - 100ul / reaction

H1PANO8

51 PAZ

185 PAZ

51 PD10

185 PD10

3146

2

2

2

2

9111:

20

20

24

20

10X dNTP

50

50

50

50

10X PCR

50

50

50

50

Taq

80

80

80

2

H₂O

375

375

371

375

DNA (cong/ful)

1

1

1

1

500

500

500

500

100ul / reaction

6

H11008/H10B4/11 PDP/PAR

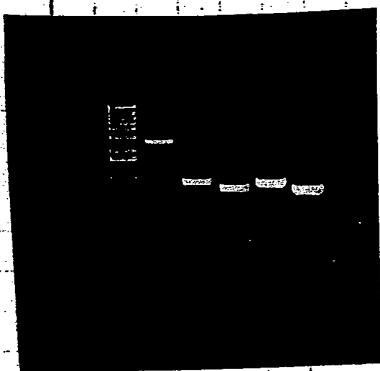
1/27/95

Run PCR:

95°C	5 min
95°C	30 sec
55°C	30 sec
72°C	1 min
72°C	7/2 min
40°C Hold	

25X

Run 5ul of Rxn on gel with 1 kb ladder



1	H10B4/11	5' Bam/3' Xba	Pst2
2	H10A008	51 bp	Pst2
3		185 bp	Pst2
4		51 bp	Pst2
5		185 bp	Pst2

Precipitate Reactions

Add equal Vol
13% PEG/NaCl

Spin 10 min

Remove Supernatant

1000ul 70% EtOH

Wash pellet

Digoxin Rinse Supernatant

Dry pellet 5 min. at RT

Redisuspend pellet in 100ul TE.

Set up Digests

DNA (PCR Rxns)	10 ul
10X #2 Buffer	5
H2O	34
Bam	0.5
Xba	0.5
	50 ul

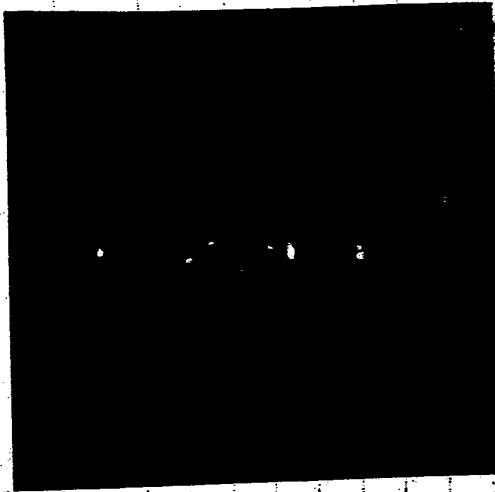
Incubate 37°C 4 hrs.
 Run on 0.8% LMP gel with
 1 kb ladder.

HTPAP08 / HTPB4II PD10 / PAZ

7

1/27/95

Cut out bands
Take picture:



1 HTPAP08 51 bp PAZ
2 ↓ 185 bp PAZ
3 ↓ 51 bp PD10
4 ↓ 185 bp PD10
5 HTPB4II PAZ

Gene Clean fragments

- Resuspend in 40ul TE

Set up ligations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
9111 + 3146	6	6						6									
9112 + 3146			6	6						6							
9113 + 3146					6						6						
9114 + 3146						6						6					
HTPB4II (PAZ)							6	6					6				
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H ₂ O	9	9	9	9	9	9	9	9	11	11	11	11	11	15	15	15	17
T4 Lig (10/ μ l)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PD10 B/ μ l					2	2								2			
PAZ B/ μ l	2	2	2				2							2			
PAZ B/ μ l 27		2	2	2				2						2			2

10X Buffer
T4 ligase
H₂O

18X
1834
18
162
12ul 12ul /Tube

Add Appropriate
of Vector Fragment
or H₂O.

3 H11008 ||| 1864||| PA2 / PDI

1/27/95

Incubate ligations 16°C overnight

16/17hr

1/27/95

1/30/95

Transform ligations

100ul of Chem Competent Cells

10ul of ligation mix

PA2 Constructs DH5 δ (3) control PA2
PDI0 Constructs M15 rep4- (3) control PDI

Incubate on ice 1 hr

Heat 42°C 45 sec

Place on ice

Add 400ul LB

Incubate 37°C off 1 hr.

Plate 200ul + 300ul onto

LB + Amp plates for all ligations
1-8 onto 150mm plates
for ligations 9-17 plates 100ul
onto LB + Amp 100mm Plates.

Incubate 37°C 0/1

1/31/95

Plates look good

No colonies on (3) Control plates

Colonies on (3) Control plates

Inoculate plates colonies into LB + Amp
for PA2 Constructs

200ul of LB + Amp in 96 well dish

Inoculate colonies into LB + Amp from

PDI0 constructs

200ul of LB + Amp in 96 well dish

HTP AND 11/17/95 B4/11 PAZ/ PD10

9

11/31/95

B+Amp:

① 9111/3146 + PAZ 1/6	48] Plate # 1
② 9111/3146 + PAZ 1/27	13	
③ 9112/3146 + PAZ 1/6	35	

③ 9112/3146 + PAZ 1/6	12] Plate # 2
④ 9112/3146 + PAZ 1/27	13	
PAZ 1/6	2	
PAZ 1/27	1	

⑤ HTPB4/11 + PAZ 1/6	48] Plate # 2
⑥ HTPB4/11 + PAZ 1/27	12	

LB+Amp/Ran:

⑤ 9113/3146 + PD10	48] Plate # 3
⑥ 9114/3146 + PD10	48	

Incubate plate 24 hrs 37°C. with aeration

Setup PCR's.

		70X		55X
9111	1	70	9112	1
3146	0.1	7	3146	0.1
10xPCR	3.2	240	10xPCR	3.2
10xdNTP	3.2	240	10xdNTP	3.2
H ₂ O	22.35	1564.5	H ₂ O	22.35
Taq	0.15	10.5	Taq	0.15
Cult.	2		H ₂ O	2
	32	30 μl/tube		30 μl/tube
		(50X)		(50X)
9113	1.0	50	9114	1
3146	0.1	5	3146	0.1
10xPCR	3.2	160	10xPCR	3.2
10xdNTP	3.2	160	10xdNTP	3.2
H ₂ O	22.35	1117.5	H ₂ O	22.35
Taq	0.15	7.5	Taq	0.15
Cult	2		Cult	2
	32	30 μl/tube		30 μl/tube

10

HTPAN08 (HTPB411) PD10 (PA2 controls)

1/31/95

HTPB411 5' Bam	0.1	65X
3' Xba	0.1	6.5
10X dNTP	3.2	6.5
10X PCR	3.2	208
H ₂ O	23.25	208
Tag	0.15	1.511.25
Cuttl.	2	9.75
	32ml	32ml / tube.

PCR.

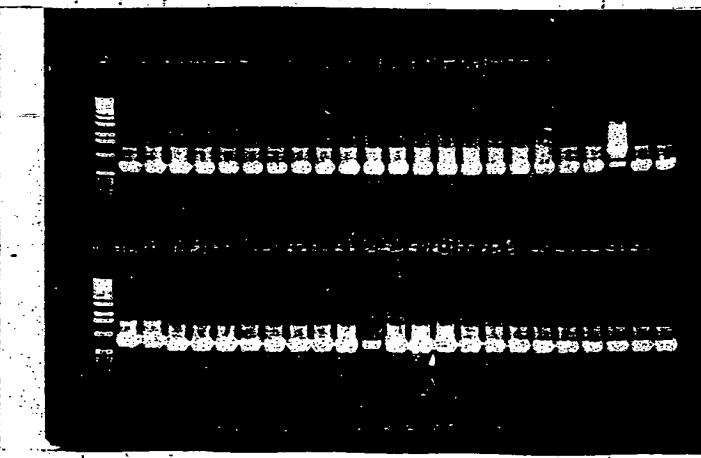
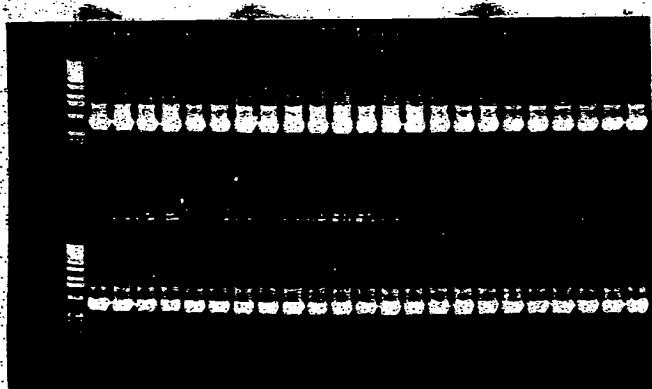
95°C	5 min	30X	PA2 controls for 9111, 9112 9HTPB411
95°C	20 sec		
55°C	20 sec		
72°C	1 min		
72°C	7 1/2 min		
4°C	Hold.		

2/1/95

Run Reactions on 1% TAE Agarose
gel with 1 kb ladder.

9113 A1-D12

9113/9114 E1-E12



HTPAN08 / HTPB4111 + PD10 / PA2

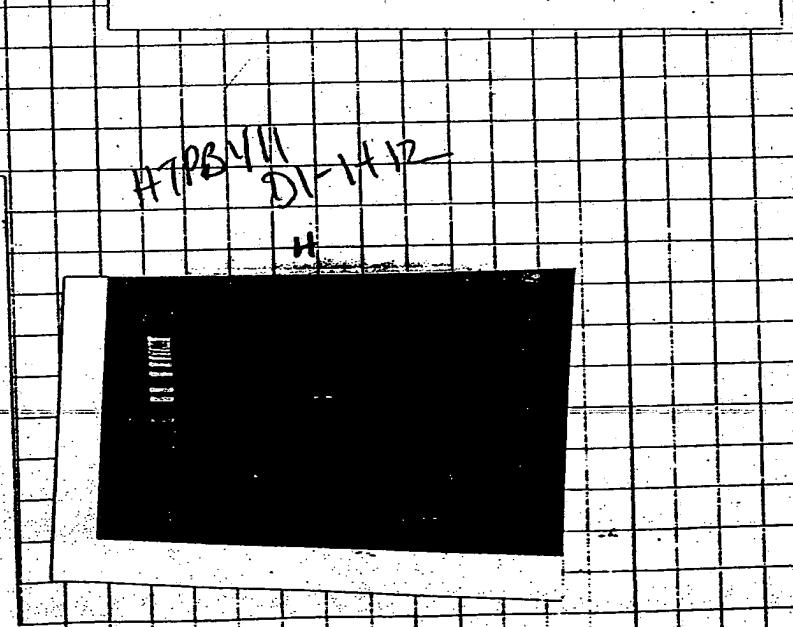
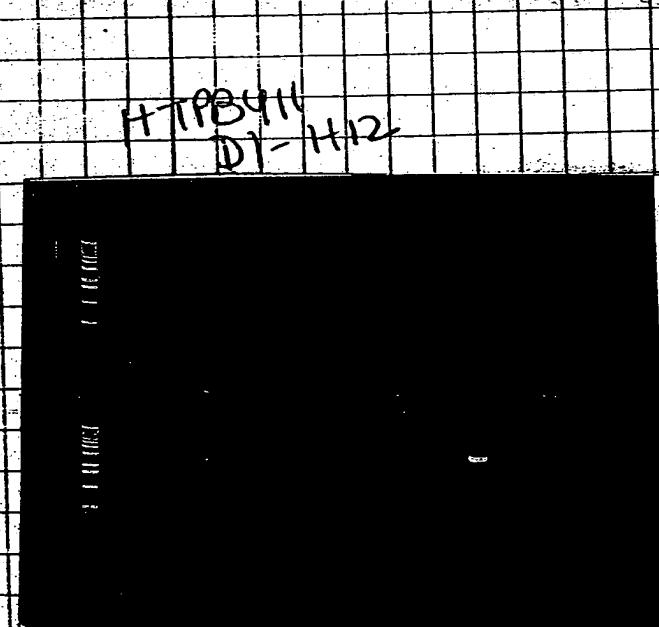
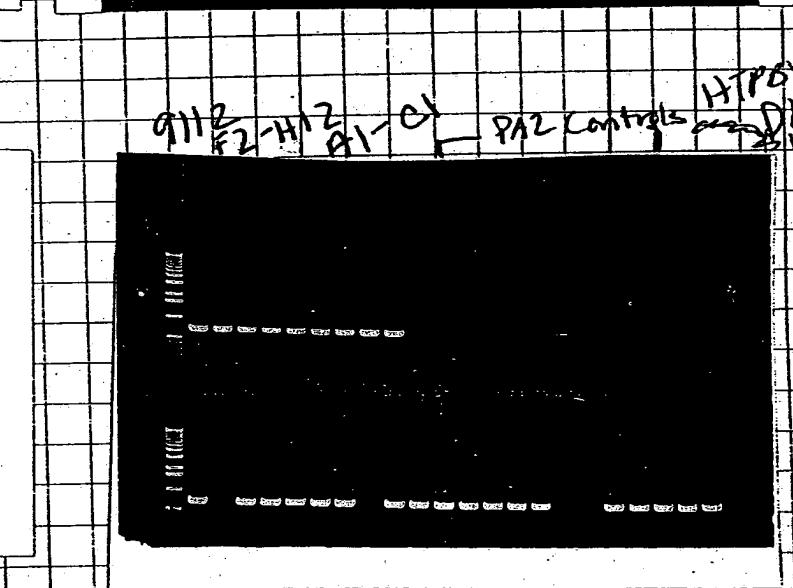
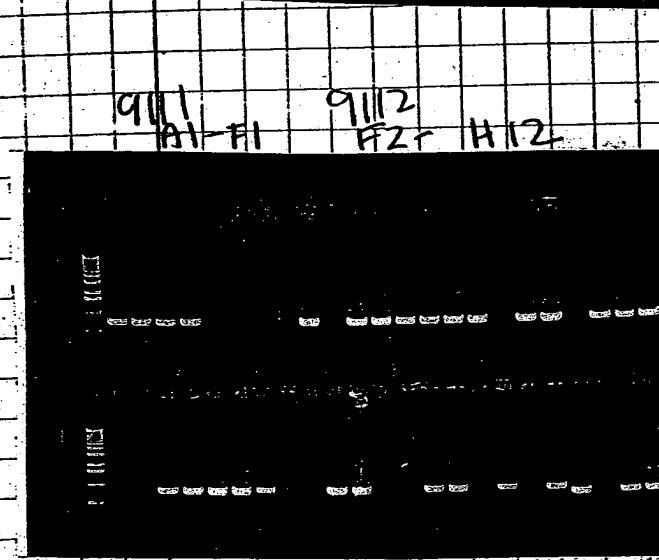
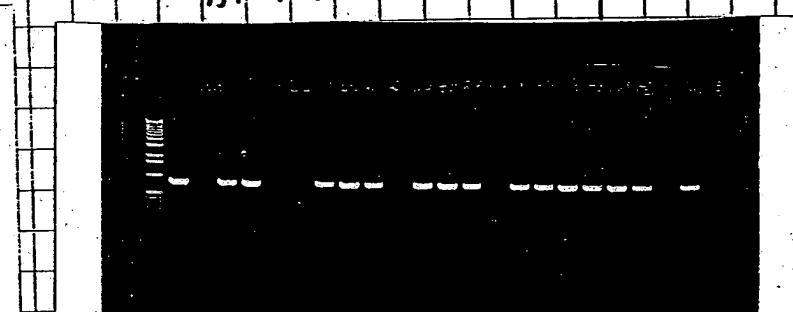
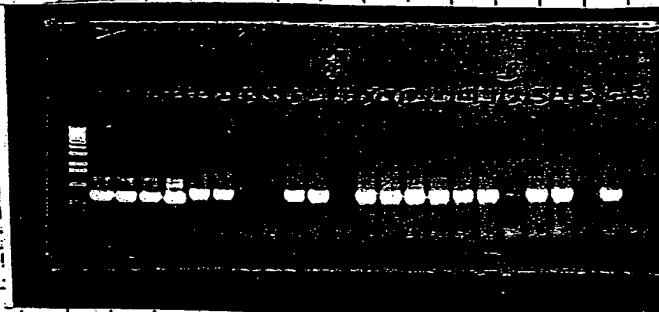
11

9112
E1-H12

9112
A1-F1

9111
A1-F1

21/195



12 HT PA 08 11 HT PB 411 PA 21 PDP

1/195

Incubate 200 ml LB + Amp/Kan
with 20ul of (P) clones to do
small scale inductions (maxes)
Incubate at 37°C w/aeration
2 hrs.

Add 5ul of 100mM IPTG to 2mM IPTG
final conc.
Incubate 4 hrs 37°C w/aeration
Spin 10min.
Resuspend pellet in 5ml H2O
Add 15ul 2X Buffer
Store -20°C till tomorrow.

Incubate 5ml TB + Amp
with PA 2 (P) clones.

(1)	-	11	9N1 131218	+ PA2 1/6
(2)	-	8	9N1 131416	+ PA2 1/27
(3)	-	10	9N2 131416	+ PA2 1/6
(4)	-	11	9N2 131416	+ PA2 1/27
(5)	-	11	HT PB 411	+ PA2 1/6

Incubate 37°C w/aeration o/n.

2/2/95

Run Protein gels 15% Stacking
15ml of Sample + 1 MW marker.

- 150V 1 1/2 hrs
- Stain 30 min 37°C
- DeStain 1 hr 37°C

HTPAN08/HTPB4/1

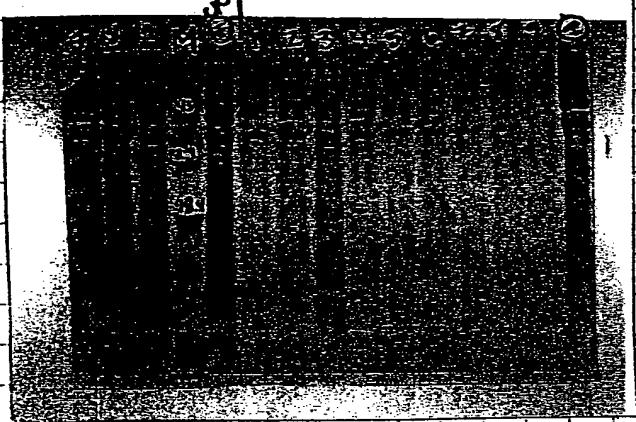
PA2/PD10

13

83 82
85 85
11 90 80

HTPAN0851 bp + PD10

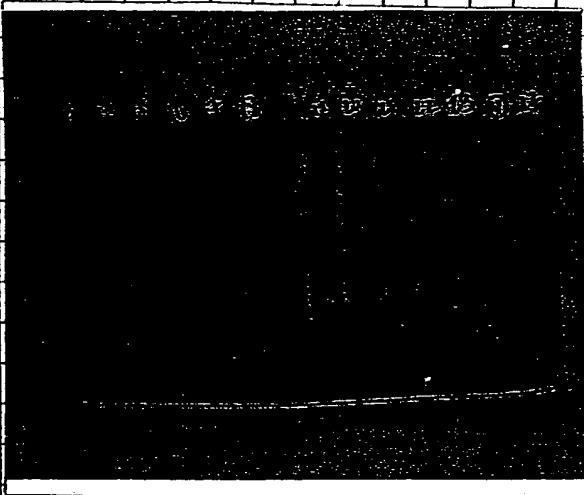
2/2/95



14

HTDANS/HTPB4/11 PD10/PA2

2/2/95



looks like
all induced
except \ominus control.

Do large scale
prep.

HTDANS/HTPB4/11 PD10

Cells

Do Bowling Mini Preps. of PA2 Constructs

2ml culture Spin 2 min

Remove Supernatant

Resuspend pellet in 750ul STET +

RNase + Lysozyme

Heat 100°C 2 min

Spin 10 min

Remove pellet

Add 750ul 13% PEG-8000/1.6M NaCl

Mix well

Spin 10 min

Remove Supernatant

Add 1ml 70% EtOH to wash pellet

Spin 5 min

Remove Supernatant.

Allow Pellet to dry at RT 10 min

Resuspend pellet in 150ul TE

Spin 2 min on 10K TFE qd with 1Kb

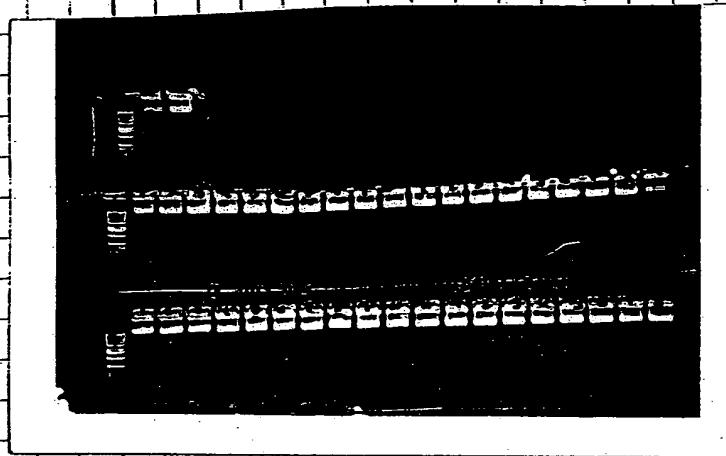
Ab Ladd

HTPAN08 / HTPB411

PD10 / PAZ

15

2/2/95



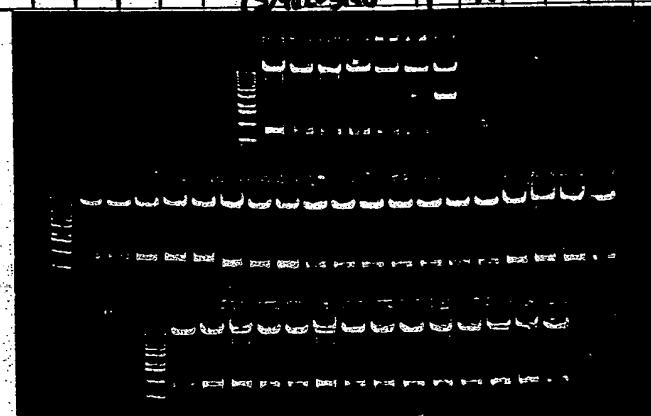
DNA 5 μl
10x 3 μl
H₂O 21.8 μl
Bam 0.1 μl
Xba 0.1 μl
3 μl

Incubate 37°C
over night

2/3/95

Run 10ul on gel with 1 Kb ladder

3791223100 | HTPAN08 / HTPB411



①② look correct
+ fast
Select ② 40 Segments
with internal
primers.

①, 2 }
②, 2 } R PD6
③, 2 } F P16
④, 2 }

HTPB411 + PAZ - FP23

Connie Checked Seq. 1 Some looked good 80 did not

16

HTPAND8/HTPB4II + PA2/ PDI0

11/3/95

- inoculate 3 ml LB + Amp w/

Cultures 1-1, 1-2, 2-1, 2-2, 3-1, 3-2

1-1, 4-2 + HTPB4II + PA2.

make Glycerole.

- inoculate 200 ml LB + Amp w/ in

HTPB4II + PA2 to do Maxi Prep

- inoculate 10 ml LB + Amp Kan with

induced culture of HTPAND8/85 bp + PDI0

(#12) - Do large scale induction

11/4/95

- made Glycerole Stocks

- 80°C Protein Expression Box #1

- HTPAND8 185 bp + PDI0 #12 -

inoculate 300 ml LB + Amp / Kan

w/ 30 ml of O/D culture

incubate 37°C 3 hrs - until $O/D_{600} = 0.4-0.6$

Add 100 μM IPTG to 7 ml - 1 ml

incubate 37°C 4 1/2 hrs w/ aeration

Spin 5K 15 min

Resuspend Pellet in ~ 30 ml of

6M GuHCl pH 8

Store at 4°C O/N.

- HTPB4II + PA2 Maxi Prep

Reagen Maxi

Spin culture 6K 20 min

Pour off Supernatant

(pg 27)

HT1080s / HT108411

27

(page)

2/16/95

HT108411 + PAZ

Resuspend pellet in 10ml of P1 + RNase

Let sit RT 5min

Add 10ml. P2 while mixing
gentl.y

Add 10ml. P3 while mixing

Let sit on ice 20 min

Spin 20 min 8K.

Equilibrate Tip-500 with 10ml QBT

Apply Supernatant to Equilibrated
Column

Wash Column 2x with 30ml
QBT

Elute DNA ca 15 ml QF

Add 0.7x (10.5ml) of Isopropanol

Mix Well

Spin 9K for 25 min

Pour off Supernatant

Wash pellet with cold 70% Ethanol
(10ml)

Spin 9K 10 min

Pour off 70% Ethanol

Allow pellet to dry at RT

Resuspend pellet in 1000ul TE

Run 1ul on gel

Read OD 260/280 at 1:200 Dilution.

abs 260.0 nm	abs 280.0 nm	bkg 320.0 nm	260.0 nm	280.0 nm
-0.0063 0.1519	-0.0028 0.0964	-0.0022 0.0232	6.9003 1.7591	0.1449 0.5685

1.52 ug/ul + 1.08
Total

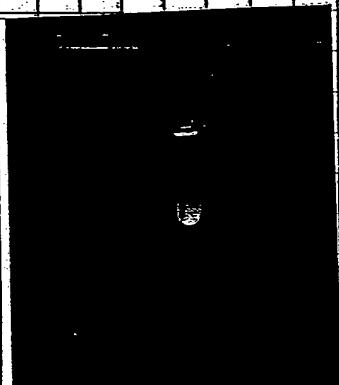
Store 24°C Plasmid Book #2.

Sequence w/ internal primers to confirm sequence

28

HTPB4/11 & HTPA1/08

2/14/95



looks good.

- See if Sequence is
good

2/15/95

Digest 1ug DNA w/Bam/Xba
to see if insert "Pops" out

DNA (250ng)	4
10X #2	3
H ₂ O	22.6
Bam	0.2
Xba	0.2
<hr/>	
	30

Incubate 37°C

Submit for sequencing w/ internal
primers. HTPB4/11/PA2 RP/FP

RP05	RP01A	RP06A	RP10	FP14	FP18	FP22 A
RP06	RP03A	RP07	RP11	FP15	FP19	FP23 C
RP07	RP04A	FP08	FP12	FP16	FP20	RP50
RP08	RP05	RP09	RP13	FP17	FP21A	

Submit for Sequencing w/ internal
 HTPAN08 51bp + PAZ
 HTPAN08 185bp + PAZ.

Clone Plasmids to Strep to submit
 to Protein Expression for bacteriavirus.

5 bp.			185 bp		
RP12	FP14	RP01	FP16	FP17	RP50.
FP13	RP05	FP18	FP14	RP01	
RP10	FP08	RP06	RP05	FP18	
FP16	FP17	RP50.	FP08	RP06	

HTPAN08PA5/ RP1/FP | HTPAN08P18 SRP/FP.

HTPAN08 185bp + PD10 #12 Large Scale
~~overnight~~ inductions.

Spin Cell Culture 20 min 8K.

Prepare NiSO₄ Column.

Apply 2ml Resin to Column
 Wash 20 ml H₂O.

Add 30 ml 0.1M NiSO₄ to Column

Wash 30 ml H₂O.

Equilibrate with 30 ml 0.1M Tris HCl pH 8.

Apply Supernatant - Collect Flow.

Wash 45 ml pH 8 - Collect pH 8.

Wash 45 ml pH 6 - Collect pH 6.

Elute 6 ml pH 5 - Collect pH 5.

Step 45 ml pH 2 - Collect pH 2.

Run on 15% Acrylamide Stacking gel.

240 μ l H₂O

20 μ l Eddi on HCl prep

7.5 μ l 50% TCA

50 μ l 0.15M NaDOC.

mix well

Spin 10 min

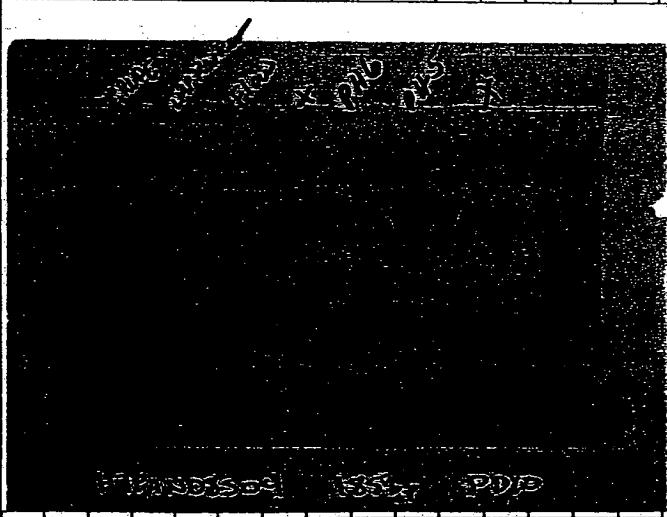
30 HTPB41 + HTPA008

2/15/95

Remove Supernatant
Resuspend Pellet 10ul 0.2M NaOAc
Add 10ul 2x Dissociation Buffer
Boil 5min - lost pH8 + pH2 Samples
mixed with water.
Spin 5min
Run 20ul on gel with 1MW
Protein marker
150V 1hour.
Stain 30min. 37°C
DESTAIN overnight.

2/16/95

HTPA008 185 P110 #12



Protein looks good

Reapply 3ml of
pH5.5 to Calcium
ride 30ml pH8
Send to Protein
Expression to
have renatured
over column

Inoculate 500ul of 1B1 Amp/kan
with 20ul of HTPB411 + P25 cell

HTPB/II + H100W081

31

Incubate 37°C w/aeration
2 hrs.

Add \approx 100 mM IPTG to 2mM
10ul.

Incubate 37°C w/aeration
overnight.

2/16/95

2/17/95

Spin Cultures 2 min

Remove Supernatant.

Resuspend culture 20ul H₂O.

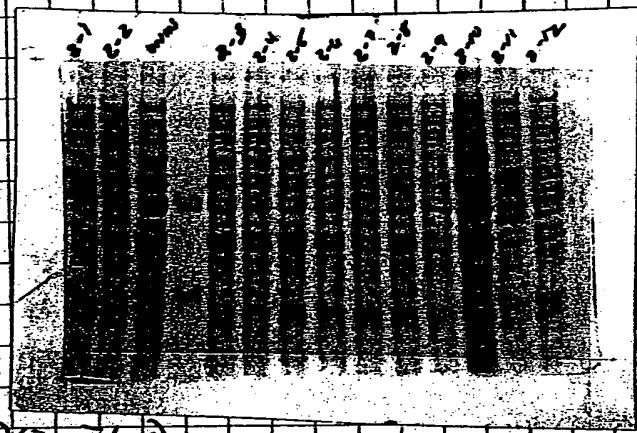
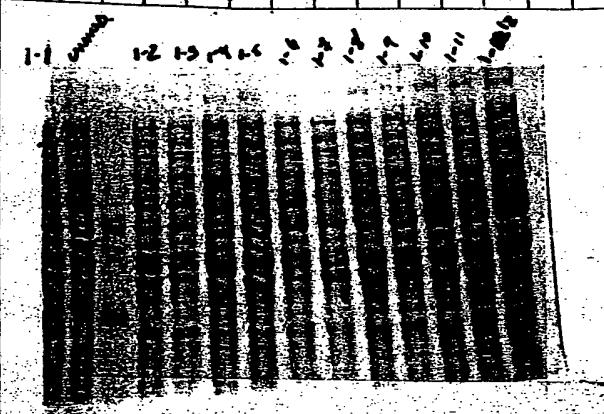
Add 2ml 2X Dissociation Buffer.

Heat 100°C 5 min

Spin 5 min.

Run 10ul on 10% Stacking
gel.

Accidentally ran 1MW Marker instead
of Rainbow Marker.



HTPB/II + PQA E(2)

Run 150V 1/2 hr

Stain
PAS

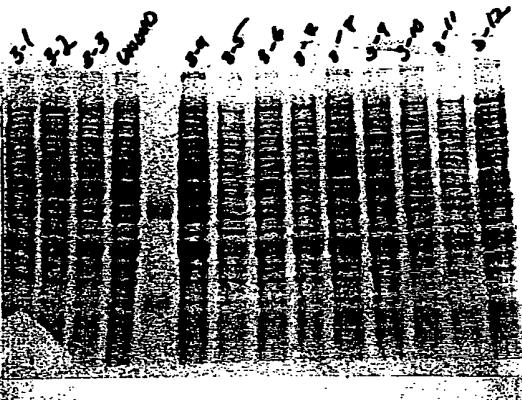
16-17 Pale

2/17/95

32

HTPAND8 / HTPB411

02/21/95



HTPB411 + PDE60

Try growing up 1 for induction -
large shake

02/21/95

Incubate 5ml LB + Amp/Kan.
with HTPB411 + PDE60.

2-3 - 3-4
Incubate 37°C

Transform - HTPAND8 51bp + PDE 9/13/95
HTPAND8 51bp + PDE60 1/5/95
using M15 Chemically Competent
Cells.

Thaw M15 on ice
to 100 μl of Cells add ligations
incubate on ice 1 hour.

02/21/95

~~1000T + SV~~
pg 26

33

2/17/95

Mix well

Spin 10min

Remove Supernatant

Resuspend in 10ul 0.2M NaOH

Add 10ul 2X dissociation Buffer

Heat 100°C 5min

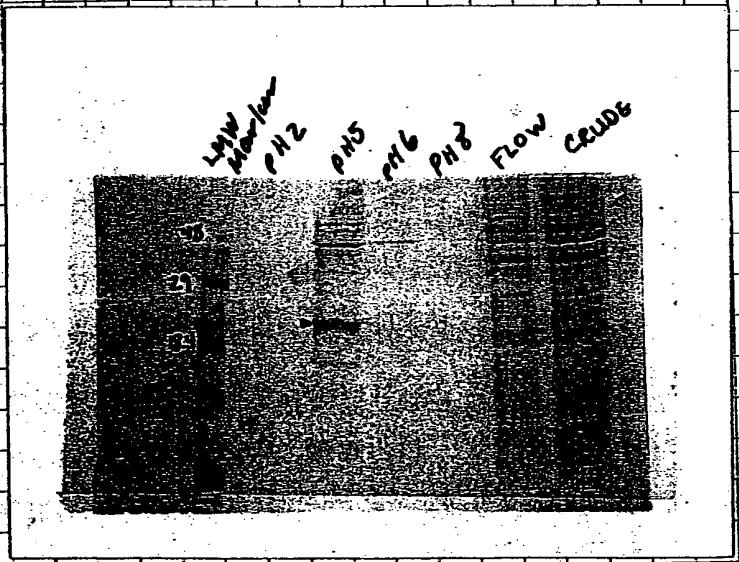
Run all on gel.

150V 1 hour

STAIN 30min 37°C

DESTAIN 30min 37°C

Take Picture.



looks like we have protein.

Looks slightly contaminated

Try all eluting
To try and clean
up prep?

Need protein PAGE

2/21/95

pick 48 more clones from yester
2/13/95 into 700 ml of 1.8Mmp/kan

HIPAN08 | HTPB411

43

(pg 32)

2/21/95

Heat 48°C 45sec.

Place on ice

Add 400ul of LB

Incubate 37°C 1 hour.

Plate 300ul onto LB+Amp 150mm plate

100ul onto LB+Amp/kan 100mm plate

Incubate 37°C O/N.

2/22/95

PCR HTPAN08 S1 + PQE60 48 BE1-H12
HTPB411 S1 + PD10 48 AI-D12
into 200ul LB+Amp/kan

Incubate 37°C w/aeration O/N.

HTPB411 + PQE60.

10 300ml LB+Amp/kan. Add
3ml of O/N culture. 2-2/3-4

Incubate w/aeration 3 hrs until
 $OD_{600} \approx 0.4 - 0.6$

Add 100ml IPTG (1) 2mM (1ml)

Incubate 37°C 4 1/2 hours

Spin cultures 5K 20min

Remove supernatant

Resuspend pellet in 30ml 1M甘油HCl

pH 8

Store 4°C O/N.

2/23/95

Do PCR of HTPAN08 Clones.

44

HTPAN086/H18B4/11

2/23/95

HTPAN08509 51bp + PD10
(50X)

9113	1.5	75
B146	0.1	5
10X dNTP	3.2	160
10X PCR	3.2	160
H ₂ O	21.9	1095
Taq	0.1	5
Culture	2	

30μl / tube

HTPAN08504 51bp + PD10
(50X)

9113	1.5	75
PQES 31	0.1	5
10X dNTP	3.2	160
10X PCR	3.2	160
H ₂ O	21.9	1095
Taq	0.1	5
Culture	2	

30μl / tube

PCR program # 10c

Run 10μl on gel w/ 1kb ladder



HTPAN08 PD10 A1-D12
A2, A7, B12, C5, C7, D5, D9 D10
Inoculate 2ml LB + Amp/kan
w/ 20μl O/N culture

HTPAN08 PD10 E1-H12

E1, E2, F1, F2, G1, G3, H1, H2
Inoculate 2ml LB + Amp/kan w/ 20μl O/N culture
Incubate 41° C 2T Overnight

HTPB111 + DDF60

45

2/23/95

HTPB111 + DDF60. 2-2 + 3-4

Spin Culture 3K 10 min.
Transfer Supernatant to fresh tube
(Crude Extract)

Prepare Column.

Prepare Column with 2ml NTA Resin

Wash 30ml H₂O

Charge 30ml 0.1M NaSO₄

Wash 30ml H₂O.

Equilibrate 30ml 0.01M GnHCl pH 8.

Apply Supernatant to Column.

Collect as flow.

Wash 30ml 0.01M GnHCl pH 8

Collect as pH 8

Wash 30ml 0.01M GnHCl pH 10

Collect as pH 10.

Elute protein to 5ml 0.01M GnHCl pH 5

Collect as pH 5

Strip column. 30ml 0.01M GnHCl pH 2

Collect as pH 2.

Store 4°C O/W.

2/24/95

HTPB111 + DDF60.

400ul H₂O

2ml of eluted Protein in 0.01M GnHCl.

50ul 0.5% NaDSO₄

75ul 50% TCA

Mix Well

Spin 5 min

Remove Supernatant

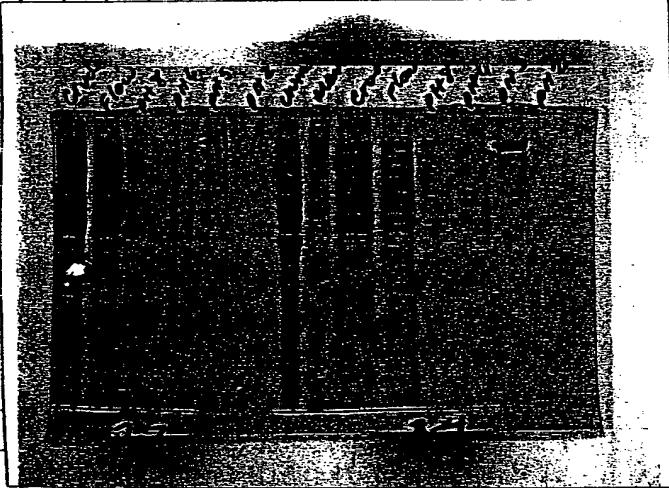
Resuspend pellet in 0.2N NaOH - 10ul

46

HTPANSB HTPBY11

2/24/95

Add 10ul 2X Dissociation Buffer
Heat 100° 5 min
Spin 1 min
Run 15ul on gel with Uninduced
and Rambow marker
12.5% Acrylamide Stacking gel
150V 1 hour



STAIN 37°

30 min

DESTAIN 37° 30 min

Take Picture

looks like isolated
protein

Store at 4°

Ask Steve about
how to get
over renature

HTPANSB 51bp + PDE/PD10

Place tubes at 37° w/ aeration
for 2 hrs

Add 100mM IPTG to 2mM + 10ul
Incubate 37° 4 hours

Spin 1 ml 2 min

Remove supernatant

Resuspend pellet in 30ul H₂O

Add 30ul 2X Dissociation Buffer

Run 15ul on gel with ~~Uninduced~~^{1mM} marker
150V 1 hour

HTPB408 (HTPB411)

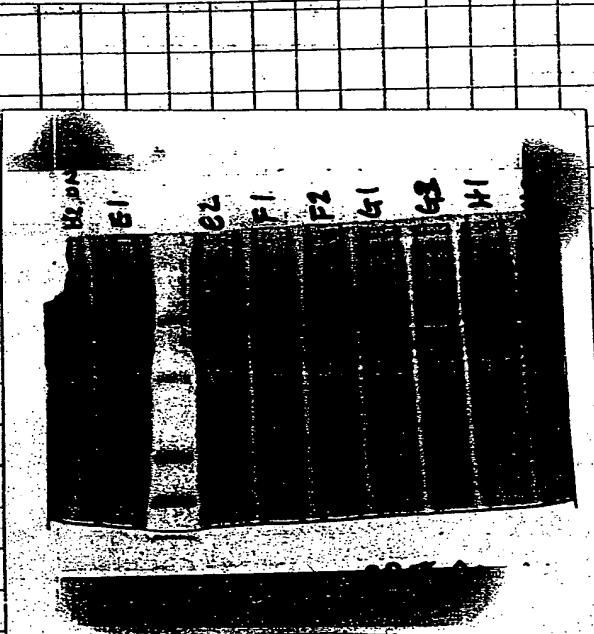
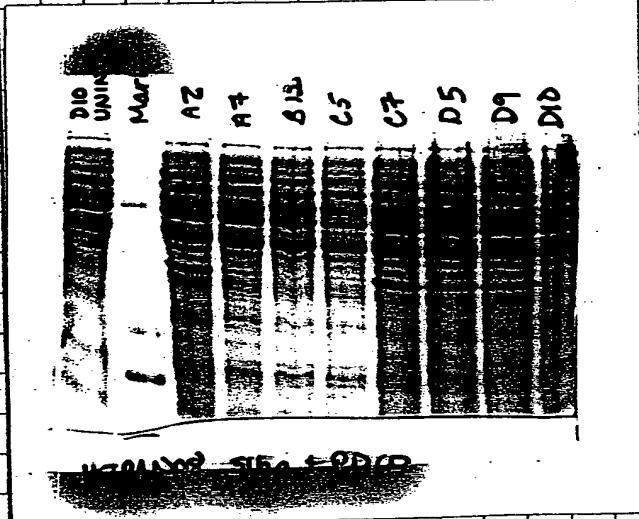
47

2/24/95

Stain 30 min 37°C
DESTAIN over weekend at RT

2/27/95

Take picture



A2
C7
C5
D9

Induced

Show up to
make Glycer.
Pick 1 to Do
Large Scale
Prep.
C7 - Large Scale
Inoculate 5ml
LB + Amp/kan w/
C7. incubate 8/w
w/ agitation. Make
Glycerols of A2, C7, D5, D9
Does not look
like anything
induced -

Try running
again subsonly
10 ml this time

48

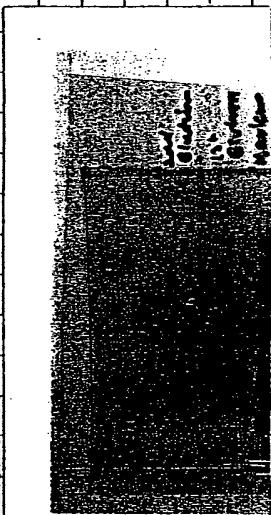
HTPANT HTP84

2/27/95

ReRun HTPN08 51bp + PGE(2)
10ul.

Run 10ul each of the 1st + 2nd
imidazole elution of HTPN08 185+PD10
#2

2/28/95



HTPN08 185 bp + PD10 #2

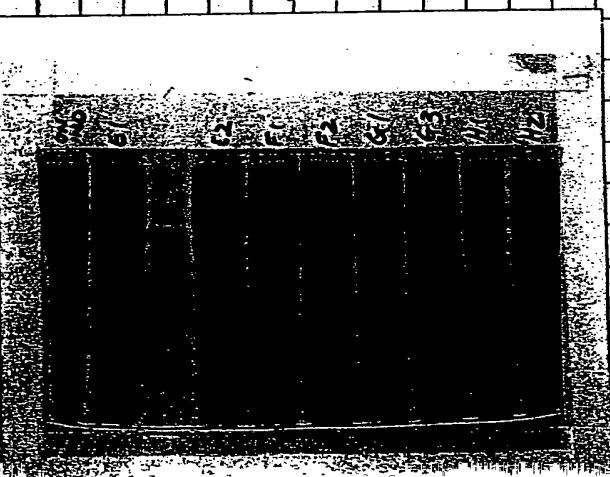
looks like both fractions
have protein.

~~HTPN08 185 bp + PD10~~

inoculate 300ml LB +
amp Kan with
HTPN08 51bp + PD10 #7
to do (Bagger max)
incubate 37° 2 (S) aeration
0'hr

ReRun HTPN08 51bp + PGE(2)

15211 1 hour
stain / DESTAIN



Does not look like
there was any
induction
very same clones
that set up

E 4,5,6,8

F 3,4,5,6

G 4,6,7,8

H- B,1,2,3,4,5,6,7

Amplification 2nd LB

HTPAN08 / HTPB411

49

Wash 7.0 ml y culture from 2/23
(Pg 44el).

Set side at RT OIN

2/28/95

HTPAN08 51bpt PD10 CT (Oxygen
max) — See Pg 42-43 for
along side

HTPAN08 51bpt PD10 CT
Large Scale induction
medium 300ml 1P37amp Kan
5 ml of OIN culture.
Incubate 37°C w/aeration
until O.D. $600 = 0.4 - 0.6 = 2\frac{1}{2}$ to
3 hours

Add 100mM IPTG to 2mM (1ml)
Incubate 37°C w/aeration
4 1/2 hours.

Spin culture 5K 15 min.

Open off supernatant.

Resuspend pellet in 30ml

10Mg HCl pH 8

Store 4°C OIN.

3/1/95

Incubate HTPAN08 51bpt POE (a)
(in 2ml 1P37amp Kan 37°C
w/aeration)

Incubate until ~ 2 hours.

Add 100mM IPTG to 2mM (4ml)

Incubate 37°C 5 hours

Spin 1ml culture

Remove Supernatant

Resuspend pellet in 40 ml H2O

Add 40 ml 2x Dissociation Buffer

Wet 100

50

H100N 11/18/84

3/1/95

Heat 100°C 5 min

Open 3 min

Run 10ml on gel w/ 24W/lane

180V 40 min

12.5% gel

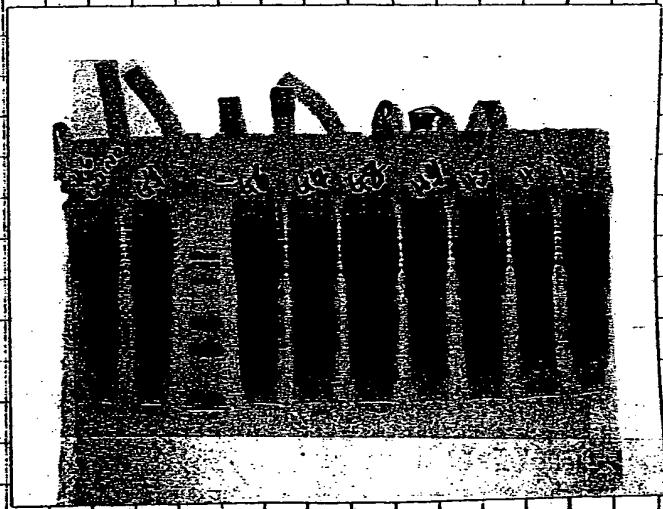
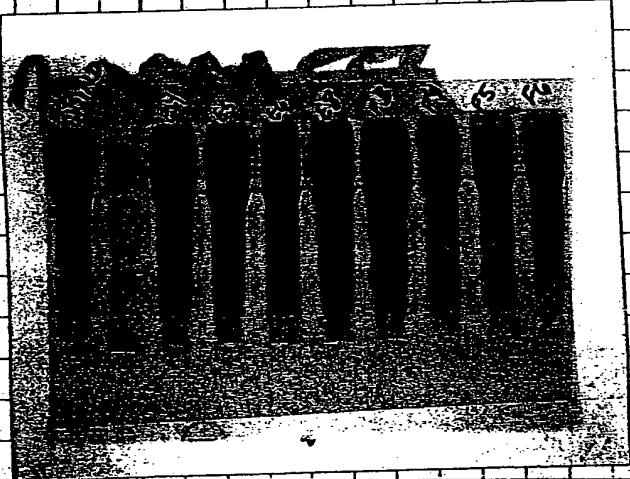
Stain O/W -

BY JAG
3/2/95

DESTAIN

DESTAIN

Take Picture



Nothing induced -

Page

60

New Clones - HTA Screen

3/7/95

Spin through G25 column.
Count well.

HTAT 33	41	1	899759.00	0.21	1.00
HTCM 40	42	1	965819.00	0.20	1.00
HT4CB44	43	1	1254146.25	0.20	0.80
HTABG 94	44	1	1446598.62	0.20	0.70

Add 10ul of Salmon Sperm DNA
Heat 100°C 5min
Quick chill.

Pour off prehyb.

Add 50ul/ml Hyb Buffer
(2x PIPES, 10% Dextranose, 50% Form.)

Add probe to hyb buffer
incubate 42°C 0/1h

3/8/95

Wash filters.

Pour off hyb
Wash filters 0.2xSSC / 0.1% SDS.

Wash filters 0.2xSSC / 0.1% SDS.

Wash 3x

Put on film

- 1-20A - HTAT 33

21-40B - HTABG 94

The others leave washing at 65°C.
Not enough cassettes.

3/9/95

Develop film.

Place remaining filters on film in
cassette Directly TAPED

W-C SV / HTPN8 Step + P0700
(pg 42) (pg 60)

61

3/1/95

Add 0.7 Volumes (sopropanol) 10.5ml.
Mix well.

Spin 8K 30 min.
Wash pellet 15 ml 80% ethanol
-20°C.

Spin 8K 10 min.
Pour off. Allow pellet to dry at RT
20 min.

Resuspend pellet in total of 400 μ l
TE and transfer to eppendorf
tube.

Read OD₂₆₀/280 - 1.200 Dilution.

Sample ID	abs 260.0 nm		abs 280.0 nm		bkg abs 320.0 nm		abs 260.0 nm		abs 280.0 nm	
	abs 260.0 nm	abs 280.0 nm	abs 280.0 nm	abs 260.0 nm	bkg abs 320.0 nm	abs 260.0 nm	bkg abs 320.0 nm	abs 280.0 nm	abs 260.0 nm	bkg abs 320.0 nm
1 μ LSV P0700	0.1502	0.0995	0.0219	1.6537	0.6047	1.5 μ g/ml				
2 HTPN8 500 P0700	0.1091	0.0715	0.0144	1.6582	0.6031	1 μ g/ml				

Run 2ul on gel w/ 1kb ladder

Plasmid looks good
stry plasmid A2.
4°C



abs 260.0 nm	abs 280.0 nm		bkg abs 320.0 nm		abs 260.0 nm	abs 280.0 nm	
	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	abs 280.0 nm	abs 260.0 nm	bkg abs 320.0 nm	abs 260.0 nm
0.0942	0.0638	0.0201	1.6947				

0.94 μ g/ml

3/1/95

Start Culture to do one induction of
HTPN8 500 P0700

Shakelet 30 ml LB Amp Kan with
C-7
Incubate 37°C ON

62 H1PANS08 51bp PD1D -

3/9/95

Incubate 300ml LB+Amp/kan
10ml of 0/1 culture of
H1PANS08 51bp + PD1D 20%

Incubate 37°C w/ aeration
fertil (OD₆₀₀ ~ 0.4 - 0.6)

Add 100mM IPTG to 2mM (1ml)

Incubate 37°C 4 hours.

Spin 8K 20min

Remove Supernatant

Resuspend pellet in 30ml 1M Gm HCl
pH 8

Store 4°C 0/1.

3/10/95

Spin culture 8K 20 min

Transfer Supernatant to fresh tube.

Run on gel.

400μl H₂O

20μg Protein in 1M HCl

50μl 0.15% NaDDC

75μl 50% TCA.

Mix well

Spin 10 min

Remove Supernatant

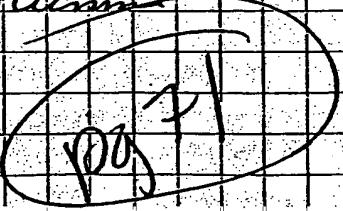
Resuspend pellet in 1ml 0.2M NaOH

Run on 2.5% gel.

Start Destar



Looks good -
Load More
Columns



HT4 Screen / HTA Screen

69

(pg 60)

3/10/94

Develop film for:

21A - 40A HT4CM40
1 - 20B HT4CB44

3/13/94

Pick Positive Clones of

HT4A7.33

HT4CM40

HT4CB44

into 20ul 5M in 96 well dish

Picks 4/8 from each clone
Incubate samples at RT 0/N

Plate HTA# for screening of
HTA B7.44

Delute 1:100 use 20ul onto
1ml 1E392 cells OD₆₀₀ = 1.0

Incubate 37°C 15 min

Plate into 150mm N24 Plates con
7ml 1B+1.75% Amp. Let set

Incubate 37°C 5 hours

Store 4°C 0/N

3/15/95

for the 4/8 clones in 8M of

HT4A7.33

HT4CM40

HT4CB44

To 50ul 1E392 OD₆₀₀ = 1.0 - add 20ul

of phage

Incubate 37°C 15 min

add 15ul N2CM broth

HTPAN 08 51bp + P.D10

71

Ag 62

3/13/95

Prepare NiSO_4 Column.

2 ml Resin

Wash 30ml H_2O

Strip 30ml 6M GnHCl pH 2.

Wash 30ml H_2O

Charge 30ml 0.1M NiSO_4

Wash 30ml H_2O

Equilibrate 30ml 10M GnHCl pH 8.

Apply supernatant - Collect flag

Wash 30ml pH 8 6M GnHCl - Collect

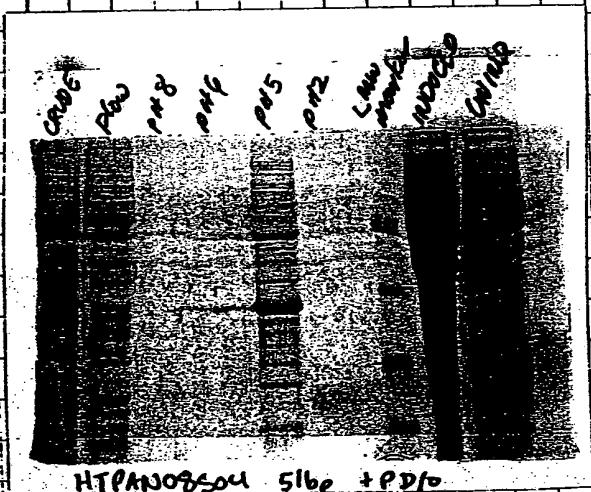
Wash 30ml 6M GnHCl pH 6 - Collect

Wash 30ml 6M GnHCl pH 5 - Collect

Wash 30ml 6M GnHCl pH 4 - Collect

Wash 30ml 6M GnHCl pH 3 - Collect 3/14/95

Run 20ul on apg with undiluted undiluted cultures.



Protein looks good

HTPAN 08 51bp + P.D10

3/15/95

Prepare 10.5% Preparation apg

1.5 mM

72 HTPANOS 86 51 bp + PDI

3/15/95

Precipitate 300 μ l of pH 5 in 160/50% TCA
Final 0.15% NaOOC
Add 100 μ l 0.1M NaOH
Add 100 μ l 2X Dissociation Buffer
Heat 100°C for 5 min
Spin 2 min
Run on gel. 100V.

Cut off Marker & Part of gel.

Spin DESTAIN



Align w/ corresponding gel.

Cut out from unstrained gel.

Place on 15ml conical
Ready gel w/ PDI addition.

133
PDI

HT4/HTA screen

75

3/16/95

HT4CB44

HT4CM40

HT4CB44

HT4CM10

HT4CB44

HT4CB44

Wash HTA + HT4 filters
0.2xSSC / 0.1% SDS. 3X 65°C
Put on film
80°C O/N.

3/17/95

Develop film

N. M. Reh
3/17/95

Random Prime Probe - HSEN37, HT4SB02, HSKB09

4/6/95

Mix by flicking
Quick Spin
Incubate 37°C 10 min

For HSEN37 use Prime it

Primers 5' 10ul
DNA 4ul
H2O 30ul
34ul

Heat 100°C 5 min

Quick Chill
Quick Spin
Add 10ul 5x dCTP Buffer
5ul $\alpha^{32}\text{P}$ dCTP
1ul Klenow

Incubate 37°C 10 min

Put through G 25 Column

Count Doses

* Did Not put HT4SB02
HT4SB02 ~~HSEN37~~ HSKB09
through Column.

CPM	2SIGX	
2391106.50	0.19	HSKBD09
3005574.25	0.19	HT4SB02
1012483.00	0.20	HSEN37

Add 100ul Salmon Sperm DNA
Heat 100°C 5 min
Quick Chill

Neat
4/6/95

Screen HOOAH/HSSEN (H5KBN5) / HT4A4 / HT4CB

4/27/95

Inoculate 30 ml TB + Amp
with HTABG94 S01, S02, S03, S04, S05 + S06
Incubate 37°C O/N.

From plated lesions - pick 6 white
colonies into each 200 ml LB + Amps
Incubate 37°C 4 hours
Do PCR.

HOOAHIC2

HSSEN37

H5KBN09

FP50	1
m13R	0.05
10X	3.2
10X	3.2
H2O	22.4
Taq	0.15
Cult	2
	32

FP50	1
m13R	0.05
10X	3.2
10X	3.2
H2O	22.4
Taq	0.15
Cult	2
	32

FP50	2
m13R	0.05
10X	3.2
10X	3.2
H2O	21.4
Taq	0.15
Cult	2
	32

HT4A480

HT4CB44

PCR Program

FP01	1.2
m13R	0.05
10X	3.2
10X	3.2
H2O	22.2
Taq	0.15
Cult	2
	32

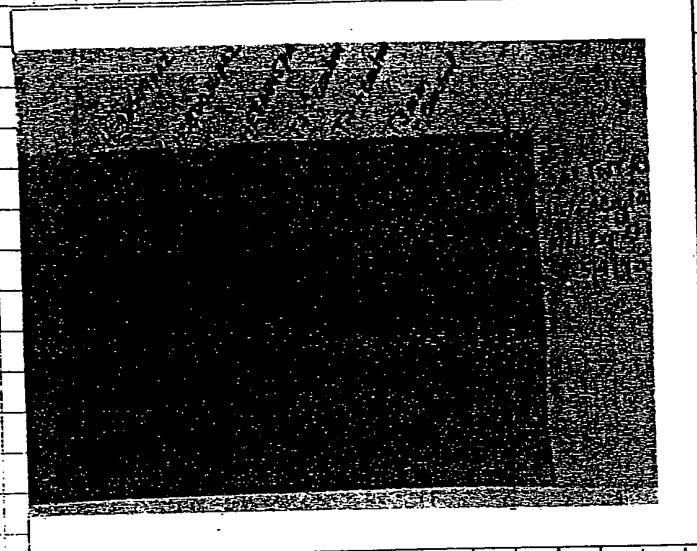
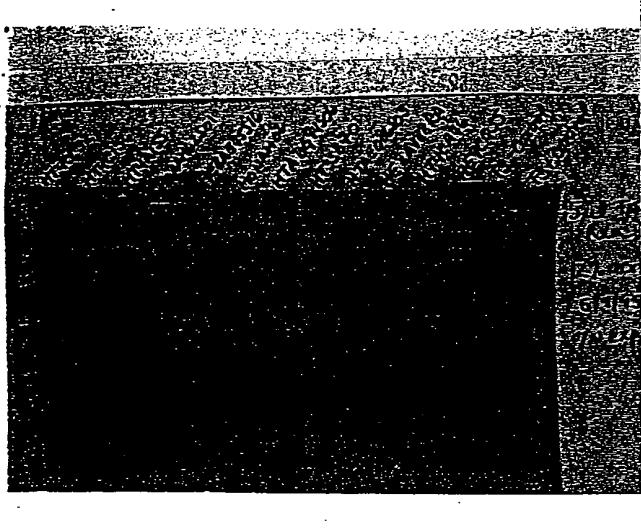
FP02	1
m13R	0.05
10X	3.2
10X	3.2
H2O	22.4
Taq	0.15
Cult	2
	32

95°C	5 min
95°C	10 sec
55°C	20 sec
72°C	1 min
72°C	1/2 min
4°C	Hold

Mark P.
4/27/95

TNT - HTPA208 51 bp Protein Prep 133

5/5/95



1972

5/8/95

Incubate LB + Amp Kan with
HTPA208 51 bp ATG in PD10
onto 100 ml.
Incubate 37°C 0/1.

Incubate 250 ml LB + Amp with
HTPA208 51 bp ATG 945000.

for Maxi Prep
Incubate 30 ml LB + Amp with
HTPA208 50000

Incubate 37°C 0/1 w/ ligation

5/9/95

Incubate 1 l of LB + Amp Kan
w/ 50 ml 0/1 culture

of HTPA208 51 bp ATG in PD10

Incubate 37°C until OD₆₀₀ = 0.4 - 0.6
Add 100 mM IPTG to 2 ml to 20 ml

134

Maxi 1A1 ABS G94506 | Midiv HOAAH62 SO2

5/9/95

Incubate 37°C 4 1/2 hours

Spin 5K 20 min

Pour off Supernatant

Resuspend pellet in a total of 100 ml

6M GuHCl pH 8
let sit 0/N at 4°CDesigen Maxi Prep
by HTABG94506
1:200 Dilution

abs	abs	bkg	abs	260.0 nm	280.0 nm
260.0 nm	280.0 nm	320.0 nm	280.0 nm	260.0 nm	
0.1548	0.1071	0.0400	1.7092	0.5851	1.55 mg/ml

Run 0.5 ml on gel.

HOAAH62 SO2. Alkeylin Lysis #1

Spin Culture

Pour off Supernatant

Resuspend pellet in pH 7.0 + RNase
(from Oligo) - 2 ml

let sit RT 5 min

Add 2 ml P2

mix gently

let sit RT 10 min

Add 2 ml P3

mix well

let sit on ice 10 min

Spin 20 min 8K

Transfer Supernatant to fresh tube

Add isopropanol 0.7 Volumes - 4.2 ml

mix well

Spin 30 min 8K

HT10808504 51b ATG + PDI0

135

Pour off Supernatant
Wash pellet 70% Ethanol
Spin 10 min 8K
Allow pellet to Dry at RT 2/1

5/9/95

HT10808502

Resuspended pellet in 1ml TE

Transfer to 2 microfuge tubes
Add equal volume 13% PEG/1.6M NaCl
Mix well

Spin 15 min

1x 70% Etanol wash

Resuspended pellet in a total of 200ul of TE

Run inl on gel -

Read OD_{260/280} 1:200 Dilution

OD₂₆₀ OD₂₈₀ OD₃₄₀ 260/280 260/340

0.0279 0.0168 0.0034 1.8322 0.5458

0.2849/1.

5/10

HT10808 51b ATG + PDI0.

Spin 8K 20 min

Transfer Supernatant to fresh tube.

Prepare NiSO₄ Column.

Pour 3ml resin into column.

Wash 20ml H₂O

Equilibrate 20ml 1M Tris HCl pH 8

Pour on Supernatant (Cricde extract)

and collect flow through.

Wash column 60ml 1M Tris HCl pH 8

Collect pH 8

Wash column - Colme 10M Tris HCl pH 10

Collect pH 6

Elute DNA Protein - 5ml 10M Tris HCl pH 5

Collect pH 5

136 HTP8N08504 51bpATG + PDI

5/10/95

Strip Column 30ml 10M Gm HCl pH 2.
Collect pH 2.

5/11/95

Run Protein Samples on 12.5% Gel
To Samples in 10M Gm HCl

450ul H₂O

50ul of Sample

50ul 0.15% Na DDC

75ul 50% TCA

mix well

Spin 10 min

Remove Supernatant

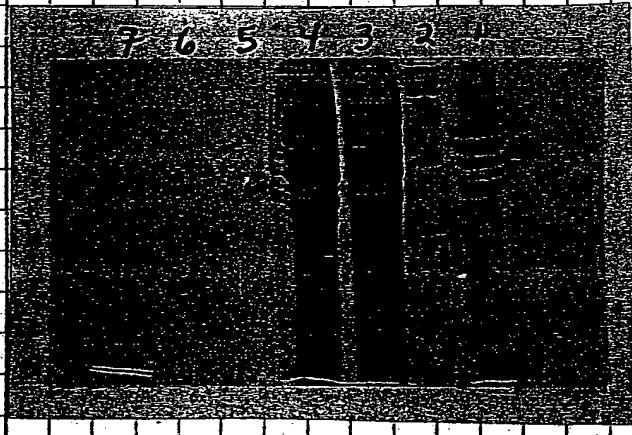
Resuspend in 10ul 0.2N NaOH

Add 10ul 2X Dissociation Buffer

Heat 0.5 min 100°C

Spin

Run Samples on 12.5% PAGE Stacking
gel. 150V 1/2 Hours



1 - Uninduced
3 - Crude Extract
4 - flow through
5 - pH 8
6 - pH 6
7 - pH 5
2 - Rainbow Marker

Does not look good try ~~several~~ ~~several~~
albume and ~~so do~~ another
induction

~~100~~ ~~100~~ ~~100~~

5/12/95

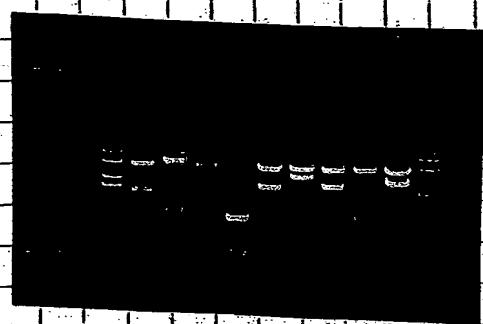
Set up Digests of unknowns to give
do Brent Kreider - He'll do
Northern.

Clone ID	Conc	5μg DNA	H ₂ O	1P X'2	Xba I	Eco RI
HSKB NO9.	2.06ug/μl	2.5	42.1	5μl	0.2	0.2
HNBA A26	0.64ug/μl	7.5	37.1		0.2	0.2
HILB430	0.5ug/μl	10	34.6			
HT4A133	PCR Product.	20	24.6			
HT4CM40	0.49ug/μl	11	33.6			
HT4CB44	1.2ug/μl	4.7	40.4			
HNFAA67	0.73ug/μl	6.8	37.8			
HT4AM80	0.34ug/μl	15.6	29			
HTABG894	0.99ug/μl	5.2	38.4			

Digest 37°C O/N.

5/12/95

Run 5μl on gel with 1kb marker
Xba I/Eco RI Digests.



- 1 - HSKB NO9.
- 2 - HNBA A26
- 3 - HILB430
- 4 - HT4A133
- 5 - HT4CM40
- 6 - HT4CB44
- 7 - HNFAA67
- 8 - HT4AM80
- 9 - HTABG894

Marked

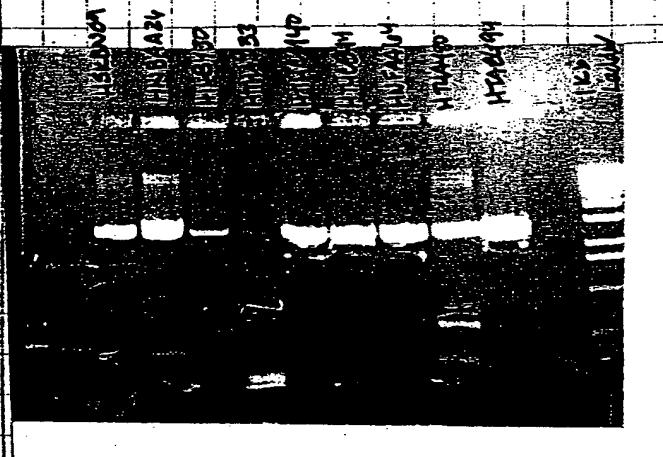
5/12/95

138

Fragment preps - Northern Blots

5/12/95

Ran on 0.8% LMP Gel with 1kb ladder.
Ran 80V 2 1/2 hours.



Cut out fragments
- place into 1.5ml
microfuge tube
Store 4°C over
weekend.

Need to digest
HTLB430 Again

ED

HTPANDS 51 bp ATG in PDVD

- INDUCTION

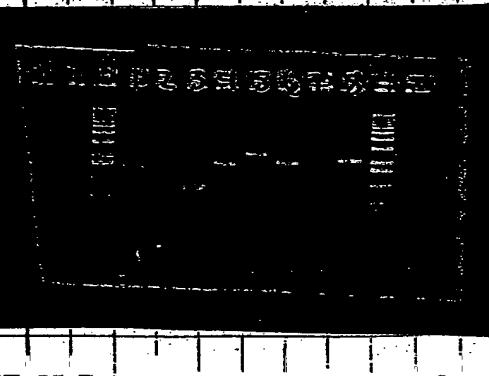
- Resuspend in 40 ml 60% Gm HCl pH 8

- Store 4°C over weekend

5/15/95

Gene Clean fragments
Resuspend in 3 ml TE.

Ran 1st on gel with 1kb ladder



1	HSKB3X09
2	HNBA26
3	HT4A133
4	HTUCM00
5	HTUB34Y
6	HNPA4G0
7	HT4A480
8	HT18G94
9	

5/15/95

Sent to Brent Kaeder - Kaid Kovacs

Clone ID	Libraries Expressed	Size in Kb Rl/Xho I	Eco	Approx. Conc(ng/ul)
HSKBN09	HT4, HSK	~1.4	AMK	~100 15ul
HNBA26	HBJ, HBM, HCA, HNB, HRG, HTO	~0.800		~100 ↓
HILBY30	HIL, HLM	~0.60		WILL SEND 5ul
HT4AI33	HT4	~0.90		~150 15ul
HT4CM40	HT4	~1.7		~150
HT4CB44	HT4	~2.5		~150
HNFAA64	HNF, HSI, HSK, HTA	~1.80		~150
HT4AY80	HT4, HTX, HT3	~0.85		~50
HTABG94	HTA	~1.7		~150
HT4CI56	HT4	~1.7		~100
HT4AI55	HT4	~1.7		~140
HT4CL32	HT4, HT5, HT3	~1.1		~150
HT4CA46	HT4, HT3, HCE, HGO, HTA, HL3, HMW	~1.7		~250 ↓
HMSAF22	HMS, HOS, HHF, HSR HTN	~2.0		~100 15ul
HT3SB70	HNF, HT3, HT4, HT5, HTA	~1.5	CLF	~300 5ul
HT4SB02	HET, HGL, HHF, HSU, HT4, HTE, HTP	~1.3	AMK	~200 10ul

Set up Digest of HILBY30 PCR product

DNA	20ul
YOD	5
H2O	24.6
Eco RI	0.2
Xho I	0.2
	50ul

Digest at 37°C
overnight

HT4SB02 51 bp A/G + PDI/O
Put over Columns
Collect Flow through
pH 8
pH 6
pH 5

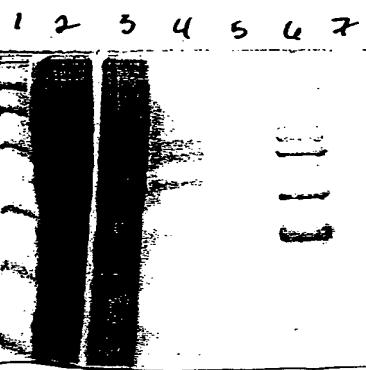
140

HTP AND SOD 516 bp A14 + PDI8

my 1360

5/17/95
7/20/95
7/21/95

Run HTP AND SOD Samples on 12% Acrylamide
gel

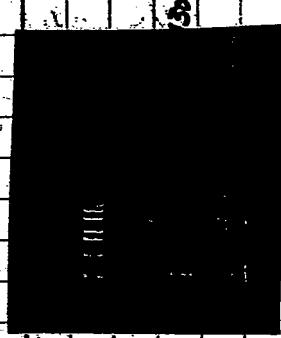


- 1 Rainbow Marker
- 2 Crude Extract
- 3 flow through
- 4 pH 8
- 5 pH 6
- 6 pH 5
- 7 pH 2.

Store at 4°C

Carrie Did Gene clean of HTZB430
Xba RI

Run gel on gel with 1kb ladder



Give to Brent
n 600 bp
~100ng gel.

H10AN028504 51bp ATG + P0D10

141

5/18/95

Grid
Reapply pH5 of H17PA0851bpATG to
fresh Column. (from 3/31/95 - pg 31)
Add 3ml pH5 + 2ml pH8.

Wash 3ml pH8.

Wash 3ml pH8.

Give to Protein expression for
renalization over column

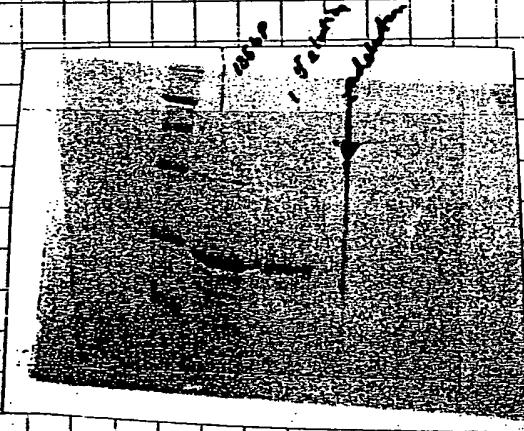
5/19 & 5/22

Computer

5/23/95

Received column back.
Elute 2ml Trisidazole elution Buffer.
2 times - Run on 12% Gel
with Marker + H17PA08135bp, ATG

51bpATG



Computer

5/23, 5/24

5/25

5/26

P0 28
P0 10
P0 10
P0 10
P0 10

5/26/95

H10AN050504 51bp ATG + P010

141

5/18/95

Grid

Reapply pH 5 of H1TPA008S1bpATG to
fresh Column. (from 3/31/95 - pg 31)
Add 3ml pH 5 + zone pH 8.

Wash Bore pH 8.

Wash zone pH 8.

Give to protein expression for
renovation over column.

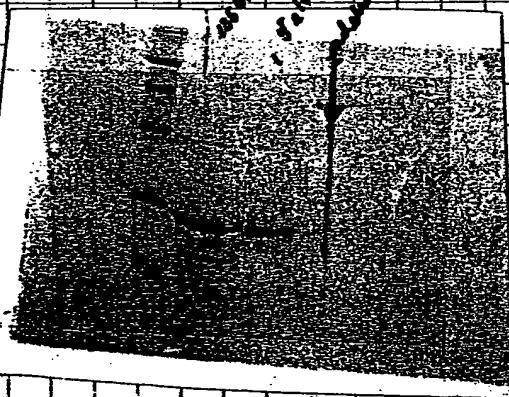
Competitor

5/19-5/22

5/23/95

Received column back
Elute 2 ml Trisidazole elution Buffer.
2 times - Run on 12% Gel
with Marker + H1TPA008S1bpATG

51bpATG



Competitor

off

5/23, 5/24

5/25

PG 28
P010
P010
MX 10

5/24/95

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